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To assess, maintain, or improve a soldier's physical/physiological/psychological capability to function effectrively under environmental and operational stress and to minimize adverse effects of stress on health safety and performance, the Pennington Biomedical Research Center performs the following six research tasks: 1) Clinical Laboratory for Human and Food Samples, 2) Stable Isotope Laboratory, 3) Nutritional Neuroscience Laboratory 4) Nutritional Neuroscience Clinical Studies, 5) Menu Modification, and 6) Metabolic Unit Project.

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FOREWORD

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For the protection of human subjects, the investigator(s) have adhered to policies of applicable Federal Law 45CFR46.

In conducting research utilizing recombinant DNA technology, the investigator(s) adhered to current guidelines promulgated by the National Institutes of Health.

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ANNUAL REPORT
US ARMY GRANT #DAMD 17-92-V2009
April 1, 1992 - March 30, 1993

Introduction

On April 1, 1992, Grant #DAMD 17-92-V2009 was awarded to Pennington Biomedical Research Center (PBRC) for to address the following **Hypothesis: Medical factors limiting soldier effectiveness can be addressed through nutritional strategies.**

The goal of this research is to assess, maintain, or improve a soldier's physical/physiological/psychological capability to function effectively under environmental and operational stress and to minimize adverse effects of stress on health, safety and performance.

Technical Objective

This research continues the research relationship between the Pennington Center and USARIEM over a five year period. Those research relationships were established under a prior cooperative agreement, #DAMD 17-88-Z-8023, "The effect of food, diet and nutrition on military readiness and preparedness of military personnel and dependents in a peace time environment."

The project allows for the continuation of the Clinical Laboratory for Human and Food Samples, Stable Isotope Laboratory, Menu Modification Project, and Nutritional Neuroscience Laboratory, all of which were initiated under Grant #DAMD 17-88-Z-8023. The project also expands the scope of research to involve Nutritional Neuroscience Clinical Studies. The project also allows for the utilization of the Pennington Center's inpatient metabolic unit for a 14 day study designed by USARIEM investigators as detailed in the Metabolic Unit Project.

The six tasks performed under this project are listed and described below.

Task 1: Clinical Laboratory for Human and Food Samples

The Clinical Laboratory performs procedures (assessment of protein, mineral, vitamin and immunologic status) to assess the nutritional status of soldiers participating in military nutrition research studies conducted by USARIEM.

Task 2: Stable Isotope Laboratory

The Stable Isotope Laboratory continues the development and field validation of stable isotope technologies to unobtrusively measure the energy expenditure of soldiers during prolonged (1-4 weeks) field exercise in extreme climates. The technology also

measures changes in body composition and body fluid status.

Task 3: Nutritional Neurosciences Laboratory

Continuation of the research in the Nutritional Neuroscience Laboratory includes multi-disciplinary studies in rats on the effect of dietary protein, carbohydrate, and caloric restriction on brain amino acid and neurotransmitter profiles, dendritic spine densities, microtubular associated proteins and dopamine D2 receptor proteins. The biochemical and morphologic variables are related to changes in behavior measured by arousal, shuttle box performance, food selection and swimming performance. The role of diet in sustaining performance under conditions of stress and rapid eye-movement sleep deprivation are studied using the above noted morphologic and behavioral parameters.

Task 4: Nutritional Neurosciences Clinical Studies

The Nutritional Neuroscience Clinical Studies are designed under the direction of behavioral psychologists to evaluate cognitive performance and man-machine interface under different dietary conditions and to evaluate nutritional intervention strategies to favorable influence mental performance in conditions of rapid eye-movement sleep deprivation.

Task 5: Menu Modification Project

Pennington Center nutritionists evaluate, through a computer data base (the Extended Table of Nutrition Values or ETNV) and laboratory analyses, the nutritional content of garrison meals. In a project conducted at Fort Polk, Louisiana, menus are modified and tested in an actual garrison setting to meet the improved Army guidelines for nutrition.

Task 6: Metabolic Unit Project

The Pennington Center inpatient 14 bed unit is made available for a 14 day research study of Special Operation Forces volunteers. The research study is designed by USARIEM scientists with collaboration and participation by Pennington Center personnel.

Military Significance and Relevance to USARIEM Needs

The Stable Isotope and Clinical Laboratory methodologies are critical components of in-house military nutrition research of the U.S. Army Research Institute of Environmental Medicine. These extramural projects provide critical capabilities that do not exist in house, but are needed to fulfill the Army Surgeon General's responsibility to provide nutritional research support to the DOD and Nutrition RDT&E Program.

The Nutritional Neuroscience Laboratory and Clinical Studies

Programs expand our knowledge of the mechanisms of action of dietary-induced alterations in behavior and cognitive function. Advances in this knowledge are the basis for developing safe and effective nutritional strategies to sustain and enhance soldier performance under conditions of environmental or operational stress.

The Menu Modification Project fulfills military needs to promote health, maintain readiness and sustain soldier performance.

The Metabolic Unit Project fulfills military need for an inpatient site for performance of specialized research utilizing the body composition assessment, energy expenditure assessment, metabolic kitchen services, and clinical laboratory expertise of the Pennington Center.

This annual report describes progress during the first year of the grant. Discussions of individual projects funded under this grant follow.

I. Clinical Laboratory for Human and Food Samples

A. Overview

The clinical laboratory for human and food samples has been making steady progress in the last year. An additional medical technologist, Gail Bley, was hired in the summer of 1992. A food chemist, Fatemeh Ramezanzedeh was hired to perform food analyses for Army projects and establish a food analysis laboratory at PBRC. Also, a phlebotomist/lab assistant, Josie Callagain was hired in the first quarter of 1993. A search is underway for an additional medical technologist and an associate lab manager.

The laboratory received three new instruments. These are two Abbott IMx immunochemistry analyzers and a Beckman Array rate nephelometer. Reagents are in for IgE on the IMx and ceruloplasmin, prealbumin, IgA, IgM, IgG, and transferrin. Methods still need to be set up for the nephelometric assays.

Dr. Tulley visited the site of the final blood collection for the Ranger 2 Study in Fort Walton Beach, Florida on October 13, 1992. Dr. Tulley also attended the meeting of the Committee on Military Nutrition Research for the report of the Ranger 2 study in Washington, D.C. on March 15, 1993.

For preparation for the Food Analysis laboratory, Dr. Tulley and Ms. Ramezanzedeh visited the USDA food research laboratories in Beltsville, Maryland on December 3-4, 1992. Colonel Askew and Patrick Dunne visited with the laboratory group at Pennington in November and offered valuable thoughts and help in the establishment of the food analysis laboratory.

A company has been chosen to implement and write software for a clinical database system in the Clinical Research Laboratory. This database, when implemented, will make logging in of samples, record keeping, and reporting of results faster and more efficient. This system will be paid for our of non-Army monies, but should benefit the Army by allowing us to provide better and more efficient service. A clinical database system manager has been hired and will begin work soon.

The laboratory has established Good Laboratory Practices and is continuing with that program. A Quality Assurance Committee has been formed and consists of personnel from the LSU Medical Center's Department of Pathology. A copy of the Good Laboratory Practices Protocol Sheet which was developed at PBRC is included in the Appendix of the third Quarterly Report (1/14/93).

B. Progress on Completed Projects

Samples which were received this year included those for the following studies: MRE study, Pikes Peak, New Generation Survival Ration Study, Ranger 1.5, Ranger 2, Ranger 2.5 and Fort Jackson Women Soldier study. Of these, the MRE study and the Ranger 1.5 study were completed. Of the others, Ranger 2 has been nearly completed and Pikes Peak partially completed. The others have been received but not analyzed yet.

Results for the Ranger 1.5 and Fort Chaffee studies are in the Appendix to the first quarterly report (7/27/92)

A method for the analysis of bromide in serum for estimation of extracellular volume was set-up using HPLC. Details of the method are included in Quarterly Report # 3 (1/14/93) (Scientific Progress, Part II). Recovery averaged 99.6% with a CV of 1.7% at 6.8 ug/ml and 1.6% at 18.9 ug/ml. Linearity was to at least 600 ug/ml.

C. Progress on Ongoing Projects

Work continues on the following projects: the final portions of the Ranger 2 study and a major portion of the Pikes Peak study (serum bromide and urine caffeine and 1-methyl xanthine). The urine nitrogens for the Pikes Peak study and the fecal and urine nitrogens for the NGSR study have not been analyzed as yet because of difficulty with our nitrogen analyzer. The company (Antek) was notified and performed an in-house maintenance on the instrument; however, we are not yet satisfied with its performance. The company is planning a second service visit.

D. Manuscripts Published/In Press

A method was developed for urinary caffeine and 1-methyl xanthine by HPLC. Details of this method are included in the

appendix of the third Quarterly Report (1/14/93). Kerrie Munson and Dr. Tulley have submitted a poster for the 1993 meeting of the AACC on the method for caffeine and 1-methyl xanthine in urine (see abstract in the third Quarterly Report 1/14/93).

Dr. Tulley presented a poster on his method for the analysis of vitamin C at the national meeting of the American Association for Clinical Chemistry (AACC) in Chicago in 1992 (see abstract in Fourth Annual Report from U.S. Army Grant #DAMD 17-88-Z-8023).

II. Stable Isotope Laboratory

A. Overview

The research conducted by the Stable Isotope Laboratory has been in the area of energy and water requirements of soldiers under harsh environmental conditions. The method used to determine energy requirements is the doubly labeled water technique, which involves oral administration of water labeled with ^{2}H and ^{18}O. Saliva and urine samples are then obtained for periods of 4-14 days, longer with re-dosing. Water intake can be determined using only the ^{2}H labeled water.

The Stable Isotope Lab was involved in several Army research projects during the current year. One was a water turnover study, part of the Pikes Peak 92 Study. For this study, urine and saliva samples were analyzed for deuterium to determine total body water at the beginning and end of the study, and for water turnover throughout the study.

Another project completed is the Rangers 92 Training Study, in which energy expenditure and water turnover were measured using doubly labeled water. There were four phases of this study, the Fort Benning phase, the Mountain phase, the Swamp phase in Florida, and a final Desert phase. The analyses for this Ranger Training Study were completed.

Discussions have been carried out regarding the collaborative study with the Israel Military services. Communications have been carried out with Major Burstein of the IDF Medical Corps, Institute of Military Physiology. Samples from the "Mountain Phase" were collected in February 1992 and have been analyzed by Dr. Andy Coward at the Dunn Nutrition Laboratories, in Cambridge, U.K. The summer study began in August, and samples were shipped to the Pennington Center for analyses of ^{18}O and ^{2}H.

B. Progress on Completed Projects

Deuterium analyses for the Pikes Peak 92 Study samples for total body water and water turnover measurements have been completed. Data were reported in the 3rd Quarterly Report (January 14, 1993) and sent to Tanya Jones for further calculations. This

data was included in paper presented at the EB93 meeting in New Orleans by Tanya Jones.

Deuterium analyses for the Ranger 92 Study samples for energy expenditure were included in the 3rd Quarterly Report. The ^{18}O data, elimination rate calculations and dilution space measurements, as well as the deuterium elimination rate and energy expenditure data are included in the 4th Quarterly Report (March 31, 1993). Preliminary energy expenditure calculations for the Rangers 92 Study were prepared for presentation at a meeting and are included in the following table. Energy expenditures during this Ranger study were very similar to those observed in the previous Ranger study. As was the problem with the first study, water turnovers were so high during the Fort Benning Phase, that the isotopes washed out quickly, causing inaccuracies in the later part of the Phase. Water turnovers have yet to be calculated.

Ranger - 1992

Fort Benning Phase	11-Aug to 14-Aug	14-Aug to 21-Aug
	4260 ± 750	4230 ± 2120
Mountain Phase	15-Sep to 19-Sep	19-Sep to 26-Sep
	5240 ± 1330	3120 ± 750
Jungle Phase	3990 ± 1100	
Desert Phase	3920 ± 360	

C. Progress on Ongoing Projects

Major Burstein of the IDF Medical Corps, Institute of Military Physiology sent samples to the Pennington Center for analyses of ^{18}O and ^{2}H. The analyses for the summer study have been completed, and those for the winter study have begun.

Discussions have begun with Col Askew regarding a water turnover study and an energy expenditure study in Summer 93.

D. Manuscripts Published/In Press

Moore, R.J., KL.E. Friedl, T.R. Kramer, L.E. Martinez-Lopez, R.W. Hoyt, R.E. Tulley, J.P. DeLany, E.W. Askew and J.A. Vogel. Changes in soldier nutritional status & immune function during the ranger training course. USARIEM Technical Report T13-92, Natick, MA: *U.S. Army Research Institute of Environmental Medicine*, September, 1992.

Jones, T.E., S.H. Mutter, J.M. Aylward, J.P. DeLany, R.L. Stephens, D.M. Caretti, D.A. Jezior, B. Cheema, L.S. Lester, and E.W. Askew.

Nutrition and hydration status of air crew members consuming the food packet, survival, general purpose, improved during simulated survival scenario. USARIEM Technical Report T1-93, Natick, MA: *U.S. Army Research Institute of Environmental Medicine*, November, 1992.

DeLany, James P., Robert J. Moore, Andrew Young, Nancy King, John Edwards and E. Wayne Askew. Energy expenditure during field training in an Arctic environment. Submitted; *J Appl Physiol*, 1993.

E. Manuscripts in Preparation

DeLany, J.P., R.W. Hoyt, E.W. Askew. Energy expenditure of unacclimatized soldiers working at altitude.

F. Abstracts

Jones, T.E., R.W. Hoyt, J.P. DeLany, R.L Hesslink, E.W. Askew. A comparison of two methods of measuring water intake of soldiers in the field. *FASEB J* 7:A610, 1993 (Abs).

Hoyt, R.W., R.J. Moore, J.P. DeLany, K.E. Friedl, and E.W. Askew. *FASEB J* 7:A726, 1993 (Abs).

III. Nutritional Neuroscience Laboratory and IV. Nutritional Neuroscience Clinical Studies

A. Overview

Drs. Berthoud, Brock, Hamdi, and Farooqui presented abstracts of their work at the meeting of the Society For Neurosciences, which was held in October, 1992, at Anaheim, California.

The Pennington Biomedical Research Center was host to Drs. Jean Kant and Robert Pleban, who presented seminars about their work for the U.S. Army. Both met with the Clinical and Basic Neuroscience teams at PBRC. Drs. Kant and Pleban offered their assessments and unofficial recommendations about our Army-sponsored project entitled "REM sleep deprivation and diet".

Members of the Clinical and Basic Neuroscience laboratories attended the Food and Nutrition Board's Committee on Military Nutrition Research workshop, entitled "An evaluation of potential performance enhancing food components for operational rations". The meeting convened at the request of the U.S. Army Research Institute of Environmental Medicine (USARIEM), and was held at the National Academy of Sciences in Washington, D.C., in November, 1992.

Drs. Brock, Hamdi, and Farooqui presented abstracts of their work at the meeting of the American Federation for Clinical Research in January, 1993, in New Orleans, Louisiana.

The Pennington Biomedical Research Center was host to Dr. Allen Rechtschaffen, who presented a seminar summarizing his decades of work in the area of sleep deprivation in rats. He met with the Clinical and Basic Neuroscience teams at PBRC, and offered his assessment and recommendations about our Army-sponsored project entitled "REM sleep deprivation and diet".

During the second quarter, Dr. William F. Waters (Ph.D. psychology; sleep and sleep deprivation) and Dr. Richard Magill (Ph.D. kinesiology; cognitive science) were hired by PBRC to work on Task 4, with Dr. Waters coordinating the effort. Two PBRC faculty members, Dr. Donald A. Williamson (Ph.D. psychology; eating disorders) and Dr. George Bray (M.D., internal medicine, endocrinology; nutrition, stress) also agreed to be part of the team. Two half-time research assistants were hired to assist in the initial stages of the project, "The Effects of Dietary Nutrients on the Consequences of Sleep Deprivation".

The team began its exploration of laboratory equipment and a laboratory site. Dr. Waters visited Bio-logic (polysomnograph hardware and software) and Puritan Bennett (the equipment distributors) in Chicago to discuss the capabilities of their laboratory polysomnograph system and possible modifications of the sleep scoring system devised for it. He also met with the PBRC architects regarding the modification of a planned apartment suite on the PBRC campus into a three bedroom sleep laboratory.

B. Progress on Completed Projects

Project: REM sleep-deprivation, stress, and food intake in the rat.

Sleep deprivation is a potent stressor in rats. They survive only 16 - 54 days during selective deprivation of the sleep stage known as rapid eye-movement (REM) sleep. Our laboratory has investigated the effects of short-term (96 hours) of sleep deprivation in the Sprague-Dawley rat, using the platform/water tank method. Our initial studies have shown that 96 hours of REM deprivation was sufficient to produce the phenomena of combined hyperphagia and weight loss in rats, as well as a decrease in their immobility time in the swimming cylinder of Porsolt.

In the next series of experiments, selected areas of the rats' brains (anterior hypothalamus, lateral hypothalamus, paraventricular nucleus of the hypothalamus, and parietal cortex) were dissected by the punch method of Palkovits, and prepared for analysis of norepinephrine (NE) levels using high performance liquid chromatography coupled to electrochemical detection. At this time, analyses of the parietal cortex, anterior hypothalamus and lateral hypothalamic nuclei are completed. All data were analyzed using analysis of variance (ANOVA) followed by unpaired Student's t-test for comparisons between groups. Significance was accepted at the 95% confidence level.

Upon analysis by HPLC, it was found that NE levels in the parietal cortex were significantly increased ($p < 0.05$) in both the TC and REMd groups, compared to the CC group (CC group, 24.78 $\pm$ 2.51 ng/mg protein; TC group, 38.35 $\pm$ 2.98 ng/mg protein; REMd group, 36.90 $\pm$ 2.98 ng/mg protein). However, parietal cortical NE levels in the TC and REMd groups were not statistically different from one another. In the lateral hypothalamus, NE levels for the CC, TC, and REMd groups were 24.73 $\pm$ 2.91 ng/mg protein, 18.84 $\pm$ 1.20 ng/mg protein, and 31.11 $\pm$ 1.84 ng/mg protein, respectively. Statistically, the CC and TC groups were not different, but the REMd and TC groups differed ($p < 0.05$). In the anterior hypothalamus, NE levels for the CC, TC, and REMd groups were 82.80 $\pm$ 9.27 ng/mg protein, 59.58 $\pm$ 4.37 ng/mg protein, and 56.32 $\pm$ 2.79 ng/mg protein, respectively. Statistically. the CC and TC groups were not different, and the TC and REMd groups were not different, but the REMd group differed from the CC group ($p < 0.05$). Analysis of the paraventricular nucleus samples are not yet completed.

The levels of NE found in the lateral hypothalamic nuclei of the REMd group correlated very well with the earlier observation that the animals demonstrated significantly increased caloric intake after 96 hours of REM sleep deprivation. From the perspective of food-intake regulation, increased NE activity in the lateral and paraventricular nuclei of the hypothalamus are known to cause hyperphagia. NE levels in the anterior hypothalamus did not correlate with the behavioral measurements in the present study.

Perhaps the most interesting observation from the HPLC analysis was the significantly increased NE levels in the parietal cortices of both the TC and REMd groups. Catecholamine activity in the cerebral cortex is known to be directly related to locomotor activity in rats. Since the rat's spontaneous locomotor activity is a reasonably accurate predictor of the animal's behavior in the Porsolt test, it was expected that NE levels in the cortex would be abnormally high only in the REMd group. Thus, it was not surprising that the NE content of the cerebral cortex of the REM deprived rats was high. However, NE levels in the TC group were also abnormally high even after 96 hours, when their behavior in the Porsolt test was closer to that of the CC group. These data suggest that performance of the rats in the Porsolt test was not directly related to NE levels in the cerebral cortex. It is interesting to note that the parietal cortex is one of the brain areas that receives NE exclusively from the locus coeruleus, and others have reported that environmental stress increased electrical activity in locus coeruleus neurons with no adaptation.

<u>Project: REM sleep deprivation and dopamine receptor binding in the rat brain.</u>

The Sprague-Dawley rats which were REM sleep deprived in the previously discussed project (96-hour REM deprivation, tank controls, and cage controls) were decapitated and their brains were

dissected. Selected areas of the brain were collected and stored at -80°C: the anteromedial frontal cortex, the cingulate cortex, the striatum, and an area of the dorsal forebrain which consisted of the olfactory tubercle and lateral olfactory tract. The brain tissue samples were processed for analysis of dopamine D2 receptor ligand binding, using liquid scintillation counting. [^{3}H]SCH-23390 was the ligand used to demonstrate D1 receptor binding, and in other samples [^{3}H]YM-09151-2 was used as the ligand to demonstrate D2 receptor binding. The non-specific binding of [^{3}H]YM-09151-2 was defined in the presence of (-)sulpiride and it constituted 3 - 6 % of the total binding.

Analysis of the striatal tissue has been completed. The cage control group demonstrated a normal D1:D2 receptor ratio of 2.12, to which the other groups were compared. The tank control groups demonstrated decreases in both D1 and D2 receptor densities (decreases in Bmax), but an increase in D1:D2 ratio to 2.92 (138% of normal). In contrast, the animals which were deprived of REM sleep for 24 hours demonstrated increases in Bmax for both D1 and D2 receptor populations, increased Kd, as well as an increase in D1:D2 ratio to 3.4 (160% of normal). The animals which were REM deprived for 96 hours showed similar increases in receptor population densities and binding affinities for both subtypes, and the D1:D2 ratio was 3.04 (143% of normal). These data suggest that alterations in dopamine receptor binding are a sensitive index which may distinguish the effects of stress from the effects of REM sleep deprivation in its earliest neurochemical events. Stress alone resulted in down-regulation of dopamine D1 and D2 receptor subtypes, whereas REM deprivation had precisely the opposite effect on dopamine receptor populations, even after only 24 hours on the small pedestal. The marked increases in D1:D2 ratios associated with REM deprivation are consistent with works of others who have observed facilitation of D1-mediated behavior in sleep deprived animals. Radioligand binding studies with the frontal cortical tissue samples have also been completed and the data are currently being analyzed.

<u>Project: The effects of REM sleep deprivation and consumption of a low-protein diet on rat behavior.</u>

The performance deficits of sleep-deprived rats in the Porsolt test and the increased spontaneous locomotor activity that they demonstrate after removal from the sleep deprivation chamber (prior to falling to sleep) suggest that 96 hours of REMd induces a high level of anxiety in the animals. Both human and animal studies indicate that stress- or anxiety-related components are among the earliest observable effects of sleep deprivation. These anxiety-related effects are important because they probably contribute to the cognitive dysfunction which is evident even with short-term sleep deprivation. Since serotonergic mechanisms in the brain are known to be involved in anxiety and other mood disorders, abnormal serotonin activity is thus implicated in the anxiety that

accompanies sleep deprivation. The apparent efficacy of a high-carbohydrate diet in the relief of stress in humans and the observation of diminished serotonin levels in the median raphe system of rats maintained on a low-protein/high-carbohydrate (low P/C) diet suggest that increased carbohydrate consumption may attenuate the detrimental effects of REM sleep deprivation by increasing serotonin levels in the limbic system. The present study was performed to test the hypothesis that rats maintained on a low P/C diet will demonstrate superior performance in behavioral testing and diminished physiological indices of stress compared to rats maintained on a normal-protein/normal-carbohydrate diet, when subjected to 96 hours of REM sleep deprivation.

Thirty-six male, Sprague-Dawley rats were obtained from commercial breeders. The animals were divided into 2 groups and placed on one of two purified diets: low-protein/high-carbohydrate (LP, 8% casein) ad libitum and normal-protein/normal-carbohydrate (NP, 20% casein) pair-fed with the LP group. After the animals were on their respective diets for 1 week, both groups were divided into 3 subgroups and introduced to the sleep-deprivation protocol: the REM sleep deprived groups (designated REMd20 and REMd8, to indicate the 20% and 8% casein diet groups) which resided in the water tank on 6.5 cm diameter pedestals for 96 hours, the tank-control groups (TC20 and TC8) which resided in the water tank on 15 cm diameter pedestals for 96 hours, receiving only controlled immersions, and the cage-control groups (CC20 and CC8) which remained in their home cages for the duration of the experiment, receiving only controlled handling. The animals continued on their respective diets while undergoing the sleep deprivation protocol for 96 hours.

Prior to their introduction to the sleep deprivation tank, the animals were tested for their adaptability to sudden stress using the Porsolt test, and were tested again every 24 hours for the duration of the experiment. Also, body weights and caloric intake measurements were made daily. After 96 hours, all the animals were placed in a commercially manufactured activity counter and their spontaneous locomotor activity was recorded for 30 minutes immediately following their removal from the water tank. The animals were then sacrificed and various body tissues (brain, kidneys, plasma) were dissected and stored at -80°C for later biochemical analysis. Adrenal gland weights were also recorded at the time of sacrifice.

One of the interesting observations made from this study was that there was no hyperphagia observed in the REMd8 group, i.e., the animals that were maintained on the 8% casein diet, and who were selectively REM sleep deprived (daily caloric intake was 256 $\pm$ 10 kcal/Kg body weight at baseline and 248 $\pm$ 17 kcal/Kg after 96 hours in the water tank). Previous studies had demonstrated hyperphagia in sleep deprived rats that were maintained on 20% casein in the diet, with a statistically significant increase in

caloric intake (134% of control). The lack of hyperphagia in the REMd8 group was most likely due to the nature of their diet, being high in carbohydrate content, which typically causes rats to become satiated quickly and consume less than their normal volume of feed per meal. In the present study, the 20% casein group was prevented from demonstrating hyperphagia because they were pair-fed to be isocaloric with the 8% casein group.

As expected, the body weights decreased in the animal groups that were exposed to the water tank, whereas body weights for the two cage control groups continued to increase normally (Table 1).

TABLE 1. Body Weights (Mean ± S.E.M.)

Group	baseline	after 96 hours
REMd20	344 ± 5	311 ± 6
TC20	336 ± 6	314 ± 9
CC20	339 ± 8	346 ± 11
REMd8	346 ± 3	310 ± 5
TC8	328 ± 9	308 ± 5
CC8	335 ± 6	340 ± 6

The weights of the adrenal glands at the time of sacrifice were not different between groups. For the CC20 group, 17.8 ± 1.2 (mg/100 gm body weight); TC20 group, 21.5 ± 1.9; REMd20 group, 20.6 ± 1.4; CC8 group, 20.1 ± 1.0; TC8 group, 19.7 ± 1.1; REMd8 group, 22.8 ± 1.1.

The performance of the 20% diet groups in the Porsolt test were similar to the results obtained from previous studies, although high variability in the data prevented the achievement of statistically significant differences. In previous studies, the TC group demonstrated an initial decrease in immobility time, but appeared to adapt to the water tank environment, and performed no differently from the CC group after 96 hours. In those studies, the REMd animals performed continually worse in the swimming cylinder as time progressed. In the present study, the REMd20 group differed only in that they increased their immobility times slightly after repeated exposure to the swimming cylinder. The results from all groups are presented in Table 2.

TABLE 2. Immobility times (seconds, Mean ± S.E.M.)

Group	baseline	after 96 hours

REMd20	134.1 ± 6.8	178.9 ± 20.1
TC20	134.6 ± 13.6	181.2 ± 23.0
CC20	158.1 ± 12.0	201.7 ± 23.2
REMd8	156.4 ± 11.9	151.6 ± 20.6
TC8	150.2 ± 8.3	168.2 ± 22.5
CC8	144.1 ± 7.5	208.3 ± 23.9

Perhaps the most interesting data from this study came from the measurement of spontaneous locomotor activity (results from all groups are shown in Table 3). After 96 hours, the REMd20 group showed an increase in locomotor activity that was statistically significant compared to the TC20 and CC20 groups.

TABLE 3. Spontaneous locomotor activity (Mean ± S.E.M.)

Group	% total time in ambulation (Mean ± S.E.M.)	
REMd20	71.04 ± 0.58*	($p<0.05$, different from TC20 and CC20)
TC20	64.58 ± 1.39	(not different from CC20)
CC20	58.92 ± 2.19	
REMd8	67.51 ± 3.17	(not different from TC8 or CC8)
TC8	65.12 ± 2.75	(not different from CC8)
CC8	65.65 ± 2.51	

It was noted that the animals on the 8% casein diet were tending to sleep in the activity counters before the 30-minute recording period was finished. It was also noted in the course of the study that the REMd8 animals had a tendency to fall from their platforms more often than the REMd20 animals. The mean number of falls for the two groups are presented below:

Group	#falls by 24 hrs	48 hrs	72 hrs	96 hrs
REMd20	0	8	13	22
REMd8	0	26	25	35

In summary, the central hypothesis of this study was incorrect, since the data suggests that feeding rats the low-protein/high-carbohydrate diet did not alleviate the stress associated with REM

sleep deprivation. The results of the Porsolt test are considered to be the critical data for this interpretation. Although the differences in immobility times between the 20% casein and 8% casein groups were not statistically different, there was a trend in the data which indicates that the combination of REMd and feeding the 8% casein diet made the animal's performance under acute stress even worse. Regarding the effects of the 8% casein diet, the greater number of times that the animals fell from their platforms during the course of the experiment and the fact that they fell asleep in the activity counters are evidence that the low-protein/high-carbohydrate diet increased the drive for sleep. The increase in motivation to sleep in the 8% casein diet animals most likely increased the level of stress associated with the REM sleep deprivation.

C. Progress on Ongoing Projects

Project: The effects of dietary tyrosine supplementation in the REM sleep deprived rat.

Tyrosine is a non-essential amino acid that is produced as an intermediary metabolite in the conversion of phenylalanine to 3,4-dihydroxyphenylalanine (DOPA), and is a precursor of the neurotransmitters dopamine and norepinephrine. There is experimental evidence from animal studies that a diet which contains 4% tyrosine by dry weight may elevate brain concentrations of norepinephrine, thus improving neurotransmission and compensating for stress-induced norepinephrine depletion in the brain. In recent years, investigators for the U.S. Army have demonstrated a considerable body of evidence that tyrosine administration may be an effective countermeasure to anxiety, mood deterioration, and other stress-related performance decrements in soldiers. Given the widely recognized physiological role of catecholamine neurotransmitters in attentional mechanisms in both humans and animals, and the empirical observations regarding its effectiveness in other stress paradigms, it is reasonable to propose that tyrosine administration may be effective in attenuating performance decrements in the REM sleep deprived rat.

The following studies have been undertaken to test the hypothesis that rats maintained on a 4% tyrosine diet will demonstrate performance in the Porsolt test, the Morris Watermaze, and spontaneous locomotion which is superior to rats maintained on a 4% valine diet or a normal (1% tyrosine, 1% valine) diet. The complete compositions of the purified diets are listed in the appendix. In the first series of experiments, male Sprague-Dawley rats were purchased from commercial breeders and divided into 9 different treatment groups: REM deprived rats on tyrosine diet (REM-T); tank control rats on tyrosine diet (TC-T); cage control rats on tyrosine diet (CC-T); REM deprived rats on valine diet (REM-V); tank control rats on valine diet (TC-V); cage control rats on valine diet (CC-V); REM deprived rats on normal diet (REM-N);

tank control rats on normal diet (TC-N); and cage control rats on normal diet (CC-N). The rats were acclimated to their respective diets for 1 week, then introduced to the REM sleep deprivation protocol for 96 hours. During the sleep deprivation protocol, body weights and caloric intakes are measured daily. Also, immobility time in the Porsolt test are measured at baseline and every 24 hours during the REM deprivation procedure. After 96 hours, spontaneous locomotor activity is measured and the rats are sacrificed by decapitation. Brains are dissected and stored at -80°C for biochemical analysis. At this point in time, about half of the behavioral data has been collected and neurochemical analyses of the dissected brain tissue is scheduled to follow.

Project: Urinary corticosterone levels in the rat as an index of performance under stress.

It is difficult, conceptually and experimentally, to separate the specific effects of REM sleep deprivation from the effects of generalized stress in the sleep deprived rat. Therefore, it is valuable to monitor changes in the neuroendocrine system that may be involved in altering the metabolism of nutrients and, in the case of circulating glucocorticoids, and may exert a direct effect on cognitive function by inhibition of glucose uptake in the central nervous system. Pre-screening a group of rats for their glucocorticoid responses to a 24-hour fasting stress will identify individuals whose hypothalamic-pituitary-adrenocortical axis are more responsive to stress than others. In the first part of this study, changes in urinary corticosterone concentrations in rats were assayed by radioimmunoassay, and each animal's endocrinological status will be correlated with its performance in two different behavioral tests of anxiety, the elevated plus-maze and the swimming cylinder of Porsolt. The data is presently being analyzed. In the second part of this study, urinary corticosterone concentrations in the rat will be used to predict the animal's behavioral performance under conditions of REM sleep deprivation.

Clinical Studies

Most of our work the third quarter was devoted to designing and equipping the sleep laboratories and developing the methodological details for the research project, "The Effects of Dietary Nutrients on the Consequences of Sleep Deprivation" (see appendix, second quarter report) and to the initial stages of designing a study to precede it: "Effects of d-Amphetamine, Phentermine, Caffeine and Tyrosine on Cognitive and Attention-Demanding Tasks During 40 Hour Sleep Deprivation" (see appendix, third quarter report).

Dr. Waters initiated the purchase of laboratory equipment (polysomnographs, computers, biological transducers and electrodes, etc.) and finalized the design of the sleep laboratory suite (control/equipment room, three bedrooms and furnishings). He

selected a suitable temporary site for the initial (pilot) stages of the research while the sleep laboratory suite is being constructed.

Dr. Magill began the development of the human performance battery after searching the literature and reviewing first hand the tests and procedures that comprise the various extant batteries. He intends to develop an optimal assessment battery, one with as many well-established tests and procedures as possible. In so doing, he has organized the published results of the effects of sleep deprivation on human performance in a comprehensive tabular format.

Dr. Williamson focused on the development of a thorough screening battery which will be completed during the fourth quarter. He has added the Structured Clinical Interview for DSM-IIIR (SCID) to the Minnesota Multiphasic Personality Inventory (MMPI) to assure compliance with the exclusion criteria. In addition, he located a side-effects checklist (Monitoring of Side-Effects System; MOSES) which will be used to assess any effects that drug side-effects might have on the experimental outcomes.

Dr. Bray reviewed the medical and pharmacological literature relevant to the use of the substances that will be ingested by the research subjects. He also has been arranging for the endocrine and immune system testing that will compromise one set of the dependent variables in each of the studies.

The Clinical Nutritional Neuroscience team spent most of the fourth quarter working out the details of the new acute study on the effects of CNS activating drugs and substances on cognitive performance during sleep deprivation that will precede the chronic study on the effects of dietary nutritional loading of neurotransmitter precursors on cognitive performance during sleep deprivation.

Dr. Waters has visited USARIEM in March, 1993 to discuss the CNS activating drugs and substances (and placebos), subject samples to be used in the study, and matters of experimental design and analysis. He consulted with Drs. Harris Leiberman, Herbert Meiselman, Edward Hirsch, Mary Mays, Irwin Taub, Patrick Dunne, and Gerald Krueger (in that order).

The pilot studies that were planned to precede the nutritional loading experiment were incorporated into an initial study that will test some meaningful hypotheses at the same time. The initial study, "Effects of d-Amphetamine, Phentermine, Caffeine and Tyrosine on Cognitive and Attention-Demanding Tasks During 40 Hour Sleep Deprivation", will test the effects on human cognitive performance during sleep deprivation of d-amphetamine (a maximal treatment contrast condition), caffeine, phentermine, tyrosine,

caffeine plus phentermine, and caffeine plus tyrosine (versus placebo; double blind, single dose design). The study will employ (and thus pilot) all of the methods and dependent variables to be used in the planned nutritional loading studies.

The polysomnography equipment arrived and was tested and set up. Instruction on the use of the equipment began. The temporary sleep laboratories have been organized and are nearly complete. An extensive cognitive performance battery was established for initial evaluation and the final battery will be established during the pilot testing scheduled for the next quarter.

Currently, the team is finalizing its protocol for submission to the LSU and the U.S. Army Institutional Review Boards and is submitting an application to the Food and Drug Administration for an Investigative New Drug (tyrosine).

D. Manuscripts in Press 1990 to Present

1. Chandan Prasad, Anwar Hamdi, Jeffery W. Brock, and Charles W. Hilton. Cyclo(His-Pro) and food intake. In: The Science of Food Regulation. George Bray, Ed., Louisiana State University Press, Baton Rouge, Louisiana, 1992.

2. Jeffery W. Brock, Shakeel Farooqui, Keith Ross, and Chandan Prasad. Localization of dopamine D_2 receptor protein in the rat brain using polyclonal antibody. Brain Research 578:244-250, 1992.

3. Masahiro Sakata and Chandan Prasad. Transient decrease in rat striatal D2 dopamine receptor mRNA level after acute haloperidol treatment. Molecular Brain Research 14:282-284, 1992.

4. Masahiro Sakata, Shakeel M. Farooqui, and Chandan Prasad. Post-translational regulation of loss of rat striatal D2 dopamine receptor during aging. Brain Research 575:309-314, 1992.

5. Emmanuel S. Onaivi, Jeffery W. Brock, and Chandan Prasad. Dietary protein levels alter rat behavior. Nutrition Research 12:1025-1039, 1992.

6. Jeffery W. Brock and Chandan Prasad. Alterations in dendritic spine density in the rat brain associated with protein malnutrition. Developmental Brain Research 66:266-269, 1992.

7. Shakeel M. Farooqui and Chandan Prasad. An antibody to dopamine D2 receptor inhibits dopamine antagonist and agonist binding to dopamine D2 receptor cDNA transfected mouse fibroblast cells. Life Sciences 51:1509-1516, 1992.

8. Anwar Hamdi, Johnny Porter, and Chandan Prasad. Decreased striatal D_2 dopamine receptors in obese Zucker rats: changes during aging. Brain Research 589:338-340, 1992.

9. Anwar Hamdi and Chandan Prasad. Bidirectional changes in striatal D1-dopamine receptor density during chronic ethanol intake. Life Sciences 52:251-257, 1993.

10. Shakeel M. Farooqui, Jeffery W. Brock, Emmanuel S. Onaivi, Anwar Hamdi, and Chandan Prasad. Differential modulation of brain neurotransmitter systems by dietary protein: dopaminergic system. In Press, Neurochemical Research, 1993.

11. Anwar Hamdi, Jeffery Brock, Keith Ross, and Chandan Prasad. Effects of rapid eye movement sleep deprivation on the properties of striatal dopaminergic system. In Press, Pharmacology, Biochemistry and Behavior, 1993.

12. Masahiro Sakata and Chandan Prasad. Age-associated decrease in serum prolactin level in male rats is unrelated to changes in anterior pituitary D_2-dopamine receptor and hypothalamic tyrosine hydroxylase or cyclo(His-Pro). In press, Neuroendocrinology Letters, 1993.

E. Manuscripts in preparation

1. Shakeel M. Farooqui, S. Ansari, and Chandan Prasad. Selective involvement of thiol groups in the binding of [^{3}H]YM09151-2 and [^{3}H]spiperone to the striatal dopamine D2 receptors. Submitted to Journal of Neurochemistry, 1993.

2. Jeffery W. Brock, Anwar Hamdi, Keith Ross, and Chandan Prasad. The effects of REM sleep deprivation on D1 and D2 dopamine receptors in the rat frontal cortex. (in preparation for Pharmacology, Biochemistry and Behavior).

3. Jeffery W. Brock, Keith Ross, and Chandan Prasad. REM sleep deprivation, hypothalamic norepinephrine, and caloric intake in the rat. (in preparation for Physiology and Behavior).

4. Jeffery W. Brock, Keith Ross, Ashley Cowart, and Chandan Prasad. Stimulus mismatch negativity in the anesthetized rat: normative data and the effects of aging. (in preparation for Neurobiology of Aging).

5. Shakeel Farooqui, Joseph LaFleur, and Chandan Prasad. Coupling of GTP-binding protein to dopamine D_2 receptor: presence of an epitope on the 110 kDa D_2 receptor complex recognized by antibody to amino terminal peptide of the Gi-beta. (in preparation for Journal of Neurochemistry).

6. Jeffery W. Brock, Shakeel Farooqui, Emmanuel Onaivi, and

Chandan Prasad. Differential effects of 5-HT concentrations in the rat brain with changes in dietary protein/carbohydrate ratio. (in preparation for Journal of Neurochemistry).

7. Emmanuel Onaivi, Shorye Payne, Jeffery W. Brock, Shakeel Farooqui, and Chandan Prasad. Nicotine and age-associated decrease in the tail-flick latency and anxiety in the rat model. (in preparation for Life Sciences).

8. Emmanuel S. Onaivi, Shorye Payne, Jeffery W. Brock, Anwar Hamdi, Shakeel Farooqui, and Chandan Prasad. Strain differences in the effects of dietary protein on avoidance behavior in the rat. (in preparation for Physiology and Behavior).

9. Jeffery W. Brock, Keith Ross, and Chandan Prasad. Maladaptive coping patterns in the rat induced by high dietary protein:carbohydrate ratio. (in preparation for Physiology and Behavior).

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1. Jeffery W. Brock and Chandan Prasad. Alterations in dendritic spine density in the rat associated with protein malnutrition. American Federation for Clinical Research, Southern Meeting, 1992.

2. Jeffery W. Brock, Keith Ross, and Chandan Prasad. Maladaptive coping patterns in the rat induced by high dietary protein:carbohydrate ratio. Journal of Cellular Biochemistry suppl. 16B:261, 1992.

3. Shakeel M. Farooqui, Jeffery W. Brock, Joseph W. Lafleur, and Chandan Prasad. Region specific modulation of rat brain dopamine levels by long term changes in the dietary protein. Journal of Cellular Biochemistry suppl. 16B:264, 1992.

4. Anwar Hamdi, Emmanuel S. Onaivi, and Chandan Prasad. Macronutrients modification alters D2 dopamine receptor properties in rat brain. Journal of Cellular Biochemistry suppl. 16B:264, 1992.

5. Shakeel M. Farooqui, Chandan Prasad, and Massarat Ali. Production and characterization of a monoclonal antibody to dopamine D2 receptor. The Endocrine Society, 1992.

6. Jeffery W. Brock, Shakeel Farooqui, Emmanuel S. Onaivi, Anwar Hamdi, and Chandan Prasad. Dietary protein modulates dopamine levels in the rat brain. Society For Neuroscience Abstracts 18(2):1516, 1992.

7. Shakeel Farooqui and Chandan Prasad. Retinoic acid mediated

induction of dopamine D2 receptor in human SHSY-5Y neuroblastoma cells. Society For Neuroscience Abstracts 18(1):660, 1992.

8. Amwar Hamdi, Jeffery W. Brock, Keith Ross, Shorye Payne, and Chandan Prasad. REM sleep deprivation and dopamine receptor binding in rat striatum. Society For Neuroscience Abstracts 18(2):995, 1992.

9. Shakeel M. Farooqui and Chandan Prasad. Retinoic acid mediated induction of dopamine D2 receptor in human SHSY-5Y neuroblastoma cells. Society For Neuroscience Abstracts 18(1):660, 1992.

10. Shakeel M. Farooqui, Chandan Prasad, and Massarat Ali. Production and characterization of a monoclonal antibody to dopamine receptor. The Endocrine Society, 1992.

11. Keith D. Ross, Jeffery W. Brock, and Chandan Prasad. REM sleep deprivation, stress, and caloric intake in rats. American Federation for Clinical Research, Southern Conference:779A, 1993.

12. Anwar Hamdi, Jeffery W. Brock, Shakeel M. Farooqui, Emmanuel S. Onaivi, and Chandan Prasad. Differential effects of 5-HT levels in the rat brain with changes in dietary protein/carbohydrate ratio. American Federation for Clinical Research, Southern Conference:779A, 1993.

13. Shakeel M. Farooqui, S. Ansari, and Chandan Prasad. Selective involvement of thiol groups in the binding of [^{3}H]YM09151-2 and [^{3}H]methyl spiperone to rat striatal dopamine D2 receptors. American Federation for Clinical Research, 1993.

14. K. N Roychengappa, Z.W. Yang, G. Schurin, S.M. Farooqui, B.S. Rabin, and R. Ganguli. Antibodies to D-2 receptor in neuroleptic naive schizophrenic patients. submitted to Society of Biological Psychiatry, 1993.

V. Menu Modification Project

A. Overview

Since 1985, nutrition initiatives have been introduced into the Armed Forces Recipe Service, the Army Master Menu and the Army Food Service Program to provide soldiers with diets lower in sodium, fat, and cholesterol. The Military Nutrition Division of the United States Army Research Institute of Environmental Medicine (USARIEM) has conducted assessments of soldiers' nutrient intakes. These studies resulted in the following nutrition related recommendations: continue revision of the Armed Forces Recipe File to reduce sodium in recipes, continue to decrease the percentage of

calories obtained from fat to 35% or less of total calories, and provide soldiers low cholesterol, low fat alternatives to eggs, and evaluate the acceptability and impact of using this approach to moderate soldiers' cholesterol intakes.

The Menu Modification Project incorporates modification of two weeks of Army garrison menus to meet the nutrition targets specified by the Army. The purpose of the Menu Modification Project is to provide healthful, nutritious menu selections which moderate soldier's sodium, fat, and cholesterol intakes.

B. Progress on Completed Projects

The primary objective of this project is actual implementation of the two weeks of modified menus at a U.S. garrison such as the facility at Fort Polk, Louisiana.

On May 5, 1992 a culinary research associate, Kevin Gilley, was hired.

Catherine Champagne and Kevin Gilley, accompanied by MAJ Cecilia Thomas, visited the Ft. Polk Installation on June 1, 1992 for purposes of future implementation of the project at that facility. A copy of the trip report can be found in the First Quarterly Report (July 27, 1992).

On June 3, 1992, the Committee on Military Nutrition Research was briefed on the plans for the new Menu Modification Project for 1992-93 at this site visit to the Pennington Center. A handout was presented to the Committee reviewing the past progress of this research and outlining future plans (for complete report, refer to First Quarterly Report, July 27, 1992).

The goals include keeping kilocalorie content of menus similar to current menus while reducing fat content, reducing fat content of menus to 30% of kilocalorie content in keeping with the Army's proposed updated nutrition standards, reducing cholesterol content of menus to no more than 300 mg/day, and emphasizing efforts to reduce sodium content of recipes/menus, which has been the most difficult task during previous work.

Committee members made several suggestions on methodology for doing garrison dining facility studies using the newly developed recipes/menus:

1) Consider intermingling new menu days into already existing menu when the study is done rather than study one full week of existing menu then immediately studying a full week of totally new menus (novelty of new menus will confound results).

2) Running the new menus several times in menu cycle, then

doing study (again to reduce the novelty impact of new menus)

3) Doing periodic acceptance tests of recipes at Ft. Polk or have Ft. Polk personnel periodically come to PBRC to test acceptance of recipes.

Catherine Champagne and Kevin Gilley traveled to Ft. Lee, Virginia and Natick, Massachusetts to meet with Army recipe developers and menu planners and tour the Quartermaster School (ACES). A trip report for these visits is contained in the First Quarterly Report of July 27, 1992.

C. Progress on Ongoing Projects

Kevin Gilley visited Ft. Polk, Louisiana from August 31 - September 4, 1992 to spend a week becoming familiar with garrison facility food preparation and support operations procedures. A detailed trip report of this visit is included in the Second Quarterly Report (September 30, 1992).

Prior to initial recipe development, all recipes were analyzed for nutritional content to confirm that they met the Army nutrition initiative guidelines for this project.

A consumer acceptability panel of 30 to 50 persons will be utilized for the final judgment process. Acceptability scores of benchtop testing will be reviewed with Natick personnel prior to continuing with further recipe development.

Benchtop panel testing was begun in October 1992. Personnel with food and or nutrition expertise were selected from staff at Pennington, LSU, and local food service establishments. A questionnaire (Second Quarterly Report, September 30, 1992) was distributed to the selected personnel to ascertain likes and dislikes. A revised hedonic food scale used with the newly developed recipes was designed with the assistance of Patty Prell, Natick, Massachusetts (Second Quarterly Report, September 30, 1992).

Currently we have a commitment from over 50 people to participate in the overall acceptability panel.

A two week menu cycle is in the development process with approximately one and a half weeks completed. To date seventy-one recipes have been developed for this project. Mary Boudreau and Marti Wennemann have been assisting Kevin Gilley in initial recipe analysis, food preparation and acceptability testing.

The following listing indicates the name of the recipe and its average score from the benchtop panel. The listing is inclusive of all recipes thus far planned for use in the project. A detailed

chart was included in the appendix of the Fourth Quarterly Report, March 31, 1993.

	Recipe	Final Score
1.	Caribbean Pone Bread	6.7
2.	Caribbean Jerk Chicken	7.8
3.	Caribbean Banana Pudding with Lemon Sauce	7.7
4.	Caribbean Pot Roast	7.2
5.	Caribbean Okra Stew	7.2
6.	Turkey Stroganoff	6.9
7.	Caribbean Rice Pudding	7.6
8.	Turkey Sausage	6.4
9.	Spanish Rice	8.0
10.	Potato Pancake	7.0
11.	Eggs Diablo	7.3
12.	Chicken and Grape Salad	8.1
13.	Chicken Sauce Picant	7.1
14.	Turnips and Greens	6.8
15.	Smothered Yellow Squash	6.8
16.	Chicken Salad	7.8
17.	Chicken Salad Dressing	8.0
18.	Mexican Corn Bread	7.1
19.	Sweet and Sour Cucumbers	6.8
20.	Breakfast Bars	7.9
21.	Oven Fried Chicken	7.4
22.	Roasted Garlic Potatoes	7.2
23.	Whitefish with Mushrooms	7.0
24.	Fish and Spinach	7.5
25.	Shrimp Stir Fry	8.1

Drs. Catherine Champagne, Ray Allen, and Alice Hunt visited USARIEM personnel at Natick on February 2-4, 1993 to further outline the Menu Modification project. In response to concerns be the Committee on Military Nutrition Research, it was decided that USARIEM will be presented with two weeks of menus in a Master Menu format in which the total percentage of calories from fat is 30% on a daily basis. The other requirements of the project, reduction in cholesterol and sodium, will be met as well on a daily basis. The contribution of each food menu item will be defined by computer analysis. Data can then be gathered to evaluate the acceptability of the menu in an actual consumption study.

The consumption study will be conducted according to USARIEM procedures. PBRC Menu Modification Staff, Champagne, Gilley, and Hunt will participate as recipe specialists, visual estimators, or in other areas as needed.

Redesign of the Menu Modification Project based on discussions at Natick:

The PBRC Menu Modification team will conduct the following

phases at Ft. Polk:

I. Demonstration of the cooking techniques and new recipe requirements for each recipe at Ft. Polk.

This will not involve an acceptability study. The USARIEM group recommended that Kevin Gilley demonstrate and be involved in the initial preparation of each recipe at the Fort Polk facility. Due to the number of new recipes, it was felt that preparation during the week, excluding weekends, would result in more soldiers available to consume the new food items. We agreed that acceptability should not be conducted at this early phase due to the presence of the novelty factor, which could affect the accuracy of the data.

II. Acceptability study at Ft. Polk conducted by Louisiana Tech faculty and students.

Following the completion of training Army cooks in preparation of the recipes, an acceptability study will be conducted at Ft. Polk. This will take place during 3 different weekly periods, again avoiding weekends. Kevin Gilley is required to be present to monitor the preparation procedures, assuring proper utilization of techniques previously demonstrated.

Louisiana Tech students, under the direction of Alice Hunt, will conduct acceptability testing and will also obtain acceptability data on other recipes used in the dining facility for comparison purposes.

TIME BLOCKS AVAILABLE FOR COMPLETION OF PHASES I AND II.

July	19-23
	26-30
Aug	2-6
	9-13
	16-20
	23-27
	30-Sept 3
Sept	6-10
	13-17
	20-24
	27-Oct 1
Oct	4-8
	11-15
	18-22
	25-29
Nov	1-5
	8-12
	15-19
	29-Dec 3

The following weeks have been chosen and would allow adequate time in between recipe introduction and acceptability testing to allow for evaluation and redesign as needed:

Phase I: Recipe Introduction, Chef Demonstration, and Preparation. Gilley and Champagne

Week 1	July 19-23 or July 26-30, 1993
Week 2	Aug 30-Sept 3
Week 3	Sept 13-17

Phase II. Recipe Preparation Monitoring and Acceptability Testing. Hunt, Gilley, and Champagne

Week 4	Oct 4-8
Week 5	Oct 18-22
Week 6	Nov 8-12

Consumption study by USARIEM personnel from Natick (Phase III):

The consumption study will be conducted by Natick personnel in a manner similar to other studies under their direction. Champagne will present to USARIEM personnel a two week modified menu in the Master Menu format, as mentioned previously. This menu will be modified to contain 30% of calories from fat, will be lower in cholesterol and sodium, and will contain recipe items developed by Kevin Gilley and tested for acceptability by the Louisiana Tech team led by Alice Hunt.

As presented by Natick personnel, the two week menu will be instituted at Ft. Polk, with the consumption study including only a 7-day window during this period. This aspect of the project will be directed by Natick personnel, assisted by Champagne, Gilley, and Hunt.

During the visit to Natick, Dr. Alice Hunt discussed protocols for acceptability testing with USARIEM nutritionists and food engineering personnel. Design of the acceptability study was agreed upon by all personnel. The PBRC biostatistician will assist in data analysis.

Dr. Hunt has prepared a preliminary draft for her proposed acceptability study. This is included in the appendix.

Dr. Ray Allen attended the Menu Modification meetings at Natick primarily to participate in nutrient database discussions involving the ETNV. Major Ron Shippee is particularly interested in aligning the Army Computerized Analysis of Nutrients (CAN) with the ETNV, anticipating the need for unity in view of continued collaborative work between USARIEM and PBRC. Allen's participation is important since he is involved in conversion of the ETNV to a PC base and developing programs that could meet specific Army

requirements for dietary assessment in field situations.

During the week of March 8-11, Gilley and Champagne attended training for a consumption study to take place at Ft. Jackson, South Carolina. These trainings were held at Natick. Gilley participated in a one-day training for recipe specialists on Monday, March 8. Champagne participated in a two and half day training on visual estimation procedures on Tuesday, March 9 through Thursday, March 11.

Catherine Champagne and Kevin Gilley will participate in the Ft. Jackson, South Carolina study of female recruits under the direction of Lt. Col. Nancy King. Plans are to arrive at Ft. Jackson on Wednesday, March 31. Orientation will take place on April 1. The study begins April 2 and continues through April 8. April 9 will be spent assembling data and completing paperwork. Departure from Ft. Jackson will be on April 10. Participation in this study will be helpful in preparing for the Ft. Polk study.

Benchtop testing and finalizing recipes for Phase I of the new Menu Modification study will continue. Nutritional analysis using the Extended Table of Nutrient Values will be used to analyze new recipes.

From previous research and planned future directions for the project, the Menu Modification Project will enable the military to enhance the Armed Forces Recipe File with versatile, healthy, and innovative new items. The focus on developing recipes meeting breakfast needs, as well as including ethnic dishes, addresses needs expressed by administrators both at the Quartermaster School and Center and at Natick.

D. Manuscripts Published/In Press

None to report at this time.

E. Manuscripts in Preparation

Two manuscripts are planned for preparation this summer. One will deal with the previous findings from the initial Menu Modification project. The second will be the result of the project comparing laboratory and computerized analyses of recipes presented at the 17th National Nutrient Databank Conference.

F. Abstracts

A paper entitled "Comparison of Military Recipes Using Computerized Nutrient Databases and Laboratory Values" was presented at the 17th National Nutrient Databank Conference, June 7-10, 1992 in Baltimore, MD by Catherine Champagne. Abstract is attached.

VI. Metabolic Unit Project

A. Overview

During the first year of the grant, activity in this project was focused on planning for the June 13, 1993 arrival of USARIEM personnel initial group of nine Special Operations Forces volunteers for an inpatient metabolic ward clinical project. This planning included scientific protocol development, approval by both Louisiana State University and U.S. Army Institutional Review Boards, as well as logistical matters for support of this project which will inaugurate the use of the Pennington Center inpatient unit. Details of this work are described in Section C below.

B. Progress on Completed Projects

None.

C. Progress on Ongoing Projects

Three visits by USARIEM led to the development of the project. On June 2-3, 1992, the Pennington Biomedical Research Center was the site of a review by the following members of the Committee on Military Nutrition Research: Robert O. Nesheim, Ph.D., Allison Yates, Ph.D., Johanna Dwyer, D.Sci., R.D., Bernadette Marriott, Ph.D. In addition, special consultants included James G. Penland, Ph.D. and other attendees, Colonel David Schnakenberg, Colonel E. Wayne Askew, Dr. Harris Lieberman, Major Mary Mays, and Major Cecelia Thomas. At this meeting initial plans were laid for development of an inpatient metabolic ward project to be developed jointly with the Pennington Center and staffed at USARIEM.

On December 2-3, 1992 members of USARIEM visited PBRC. Visitors included Colonel E. Wayne Askew, Ms. Tanya Jones, R.D., Ms. Catherine Gavaree, R.D., Major Ronald Shippee, and Dr. Patrick Dunne. At this visit plans for the metabolic unit project were discussed. The research project was determined to be designed by USARIEM scientists with a co-investigator appointed from the PBRC faculty. The Pennington Center committed its 14 bed inpatient metabolic unit for June-July, 1993. The project goal would be to evaluate the metabolic and physiologic status of selected volunteer subject members of the Special Operations Forces with the goal of evaluating feasibility for developing individualized diets for the special forces according to individuals and mission. Special Forces subjects were proposed to be housed for a total of 30 days at the PBRC metabolic facility. The subjects were proposed to complete physical and mental performance trials while undergoing metabolic and biochemical assessment.

On April 2, 1993, Ms. Tanya Jones and Colonel E. Wayne Askew visited the Pennington Center. The project plans were finalized through correspondence and conversations between USARIEM personnel

and Pennington Center scientists. The project to be accomplished under this task is "Assessment of intra- and inter-individual metabolic variation in special operations for soldiers." The principal investigator for the project is Ms. T. E. Jones, affiliated with the military nutrition division at USARIEM. Co-investigators are C. Gabaree, Lt.C. Murphy, Donna H. Ryan, M.D., E. Brooks, R.N., M.N. The project will involve 18 patients that will be housed in the inpatient metabolic unit of the Pennington Center.

The purpose of the study is to evaluate a group of Special Operations Forces (SOF) to determine the metabolic variation during rest, exercise, and post-exercise recovery of the individual soldiers. The underlying hypothesis is that significant variation between individuals with regard to substrate utilization will indicate different nutritional requirements for optimization of physical performance, particularly during mission deployment.

The military relevance of the project addresses the concept of development of a modular operational ration which could be reconfigured to meet the specific daily nutritional need of each SOF soldier based upon metabolic requirements and/or specific mission scenario.

The protocol can be found in the 4th Quarterly Report (April 15, 1993). The appendix contains a draft protocol. Eighteen healthy male SOF soldiers, 18 years or older, and two cohorts will be evaluated in the Pennington Center metabolic unit. These activities will take place during a two week period beginning June 13 and a two week period in July. The subjects will be volunteers who will give informed consent for the study and will be medically screened by a physician prior to testing. Those volunteers over age 35 will undergo a diagnostic graded exercise test.

In order to simulate what an SOF soldier might experience during a mission, the test diet will be limited in carbohydrates to four grams per kilogram of body weight or approximately 300 grams per day. During the study period (Days 1-11) subjects will follow a highly controlled metabolic diet. The diet plan will be a three day menu rotation of hot palatable food that will be isocaloric with each subject's estimated energy requirement. Test diets will be designed using the Extended Table of Nutrient Values (ETNV).

A duplicate portion of the subject's food intake for each day of the three day menu cycle will be prepared and blenderized. A one pound sample of this aliquot will be placed in a clean one pint glass jar and frozen for analysis. Composition analysis of each diet will be performed at the U.S. Army Natick Research Development and Engineering Center. An analysis will include protein (total nitrogen), fat and carbohydrate. (See protocol for methodology.) Subjects will be housed at the Pennington Center for 13 days. Each volunteer will be studied for 11 days. Subjects 1-3 will start on Day 1, Subjects 4, 5 and 6 on Day 2, and so on until all nine

subjects are under study.

On Day 1, height, body weight, body composition by DEXA, somatotype, muscle fatiguability and physical performance will be measured. Body weight will be measured every morning after voiding throughout the study. In addition, subjects will start the control diet and begin the 24 hour urine and fecal collection. Metabolic determinations will be carried out on blood, urine and feces and are defined with input from the PBRC clinical laboratory.

Days 2, 3, 5, 6, 8 and 9 are rest days. The daily routine on these days is less rigorous than on experimental exercise days. On these days resting energy expenditure will be obtained and subjects will engage in two 60 minute exercise sessions (cycling and treadmill walking). Intensity will be continuously monitored by heart rate response.

On Days 4, 7 and 10, each subject will perform prolonged, sub-maximal treadmill exercise in a laboratory setting. The experimental exercise protocol will occur in two sessions, separated by a six hour rest period. Blood samples will be acquired before, during and after the exercise session. Respiratory data will be collected at approximately 20 minute intervals during exercise and recovery. Additionally, a dietary manipulation will accompany afternoon sessions of the protocol. Each subject will be randomly assigned to each of three experimental trials. S1 (Supplement 1) will be the placebo trial. For the S2 (Supplement 2) trial the subject will consume a carbohydrate feeding (glucose polymer) immediately after the morning exercise. The S3 (Supplement 3) trial will provide a carbohydrate feeding both immediately after the morning exercise and during the afternoon exercise. Estimated energy expenditure will be approximately 4300 kilocalories per day. On Day 11 body composition will be determined using the DEXA.

D. Manuscripts Published/In Press

None.

E. Manuscripts In Preparation

None.

F. Abstracts

None.

APPENDIX

MENU MODIFICATION PROJECT

ABSTRACT FORM

All abstracts must be received in final form no later than April 1, 1992

Seventeenth National Nutrient Databank Conference
Baltimore, Maryland
June 7-10, 1992

Date March 19, 1992 Signature Catherine M. Champagne

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(Choose one)

☐ Oral Presentation ☐ Poster ☒ Either

COMPARISON OF MILITARY RECIPES USING COMPUTERIZED NUTRIENT DATA BASES AND LABORATORY VALUES. Catherine M. Champagne, Doris E. Sherman, Carol Baker-Fulco, and James L. Dean, Pennington Biomedical Research Center, Louisiana State University, Baton Rouge, Louisiana.

Computerized nutrient analyses using the Extended Table of Nutrient Values (ETNV) and the Army Computerized Analysis of Nutrients (CAN) were compared to laboratory (LAB) values for nine recipes (six mixed-dish entrees, two vegetables and one dessert). In addition to comparing laboratory results with computerized nutrient evaluations, determining the variation between the two data bases in analyzing the recipes was also investigated. The variables included were kilocalories, macronutrients, moisture, cholesterol, ash, seven minerals and five vitamins. The data were analyzed by a randomized block ANOVA and means compared using Duncan's Multiple Range Test. Significant differences ($p < 0.05$) were found in ash values between ETNV and LAB and potassium and riboflavin levels between CAN and LAB. Although there was a difference in CAN and ETNV for riboflavin, the potassium and ash values of CAN and ETNV were similar. While some discrepancies were noted in calorie levels and a few other nutrients within some of the recipes, they were not of statistical importance. Despite the disparities, it still appears appropriate and a more economically feasible approach to utilize computerized nutrient analyses to assess intakes of the population.

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